

Amendments to the Specification:

Please add the following new paragraph after the paragraph ending on line 27 of page 8:

-- Figures 31A-31C show the implantation of a glucose sensor in a mouse.--

Please replace the paragraph beginning at page 10, line 2, with the following rewritten paragraph:

-- The general meaning of the following terms as used in the present application, unless specifically modified, are: "Normal Cells": biological cells derived from living organisms, and/or tissues, which retain a normal genotype and phenotype, usually obtained directly from tissue or from primary culture. "Mutant Cells": biological cells with spontaneously altered genotype and phenotype, such as cancer cells, cell derived from naturally occurring genetically deficient organisms, usually obtained in secondary culture and or continuous cell lines. "Engineered Cells": genetically or chemically modified biological cells (usual original source is Normal or Mutant cells). "Transgenic Cells": biological cells derived from transgenic animals, in which the cells have genetically induced alterations of genotype and or phenotype. "Gene Transfer Cells": biological cells that have altered phenotype resulting in alteration of cell structure and or function. This includes knockouts, knockdowns, "over-expressors" etc. "Chemically Modified Cells": biological cells in which membrane, cytoplasm structural or enucleolar elements of the cell are altered permanently or for extended periods, thus altering cell structure and or function. "Artificial Cells": biological cells lacking the ability to replicate but capable of sensing and responding to their microenvironment. For example enucleated cells, or cells lacking a nucleus (e.g. red blood cells), in which genetic elements such as DNA, RNA, viral vectors, nanodevices or nanomaterials can be incorporated for in vivo uses. Hybrid Cells: biological cells that are the result of cells fusion, and or combinations of engineered and or artificial cells. "Matrix material": complex heterogeneous networks of insoluble macromolecules such as glycoproteins,

carbohydrates, structural proteins (e.g. collagen), as well as bound proteins and factors. These matrices contain specific binding sites for cells, factors (e.g. cytokines and growth factors) and proteins, which directly control cell adhesion and function *in vivo* and *in vitro*. "Biological Matrices": matrices obtained from organisms, tissues, or cell. Examples of biological matrices include interstitial matrices, basement membrane, fibrin clots. Interstitial matrices are generally composed of fibrillar and nonfibrillar collagen, elastin, fibronectin proteoglycans, hyuronate, as well as other components. Basement membranes are composed of nonfibrillar collagen (usually IV), laminin, heparin sulfate, proteoglycan, and other glycoproteins. Fibrin clots are complex networks of plasma proteins including fibrin(ogen), fibronectin, glycoproteins, ~~heparin~~heparin, thrombin collagen, as well as other plasma proteins cross-linked to the fibrin clots via Factor XIII. Additionally, fibrin clots have extensive binding sites for various factors and cells including leukocytes, fibroblasts and endothelial cells. "Engineered Matrices": genetically and or chemically modified biological matrices. "Hybrid matrices": combinations of biological, engineered and or artificial matrices. In addition, the meaning of various abbreviations as used within the present application, unless specifically modified, include ES, embryonic stem cell; MSC, mesenchymal stem cell; MAPC, multipotent adult progenitor cell; HSC, hematopoietic stem cell; NSC, neural stem cell; NPC, neural progenitor cell; MDSC, muscle-derived stem cell; ECM, extracellular matrix; EGF, epidermal growth factor; LIF, leukemia inhibitory factor; SCF, stem cell factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; ~~growth factor~~growth factor; VEGF, vascular endothelial growth factor; BMP, bone morphogenetic protein; BDNF, brain-derived neurotrophic factor; NT, neurotrophin; CNTF, ciliary neurotrophic factor; bFGF, basic ~~fibroblast growth~~fibroblast growth factor; TGF- β , transforming growth factor-beta; IL, interleukin; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IGF, insulin-like growth factor; RA, retinoic acid; and FBS, fetal bovine serum.--

Please replace the paragraph beginning at page 14, line 30, with the following rewritten paragraph:

--In one embodiment of the invention, the TRM 20 includes, for example, agent(s) that control tissue growth; tissue differentiation; tissue injury; innate immune responses; ~~acquired~~acquired immune responses; humoral immune responses; cell mediated immune responses; inflammation; acute inflammation; chronic inflammation; wound healing; regeneration; tissue repair; neovascularization; bone destruction; bone injury, repair and or regeneration; connective tissue destructions; controls connective tissue injury, repair and regeneration; fat tissue injury, repair and or regeneration; neurologic tissue injury, repair and or regeneration; and/or responses using TRM 20. The TRM 20 may include: cell to cell protein transporter molecules; antibodies; proteins, modified proteins and/or recombinant protein; chemicals; drugs; genetic elements; recombinant DNA; RNAs, including siRNA; altered RNAs; genetically altered RNAs; chemically altered RNAs; DNA; altered DNAs; carbohydrates; lipids and fatty acids; radiation energy; magnetic energy; viruses; single or double stranded DNA; and/or single or double stranded RNA.--

Please replace the paragraph beginning at page 22, line 2, with the following rewritten paragraph:

--In one embodiment of the invention, a gene transfer system is included wherein a genetically engineered cell suitable for use in the ATS is produced. For example, as experimentally shown, a Rous Sarcoma Virus Vector Model for Gene Transfer was created wherein a helper-independent retroviral vector, RCAS, derived from Rous Sarcoma Virus (RSV) was used for gene transfer in the *in vitro* and *ex ova* CAM model studies. A mouse VEGF gene (mVEGF), genebank number M25200, said genebank disclosure incorporated fully herein by reference and associated with the sequence as ~~shown~~shown in Figure 22, was inserted into the RCAS proviral plasmid vector in both "sense" and "antisense" orientations using standard recombinant DNA manipulations. Specifically, a 908 bp Taq I fragment containing the mVEGF open reading frame was mobilized from pBSK+mVEGF and ligated into the unique Cla I site of the RCAS-BP(A) proviral vector plasmid. The ligation products were screened by restriction mapping and both sense and anti-sense orientations were obtained. The resulting mVEGF and anti-

mVEGF proviral DNAs were transfected into DF-1 chicken fibroblast cells using lipofectamine, and the cultures were passaged for two weeks to allow viral replication.--

Please replace the paragraph beginning at page 23, line 24, with the following rewritten paragraph:

--In one embodiment of the invention a determination of cell viability and growth of cells incorporated into a matrix material ~~composed~~composed primarily of fibrin can be made. As experimentally shown a naturally occurring matrix (fibrin) was used to investigate its utility to entrap cells and still allow cell viability. In addition, the formation of fibrin clots served as a matrix to keep the cells localized, which is important for later *in vivo* investigation where target gene delivery is an important issue. Briefly, equal volumes of human fibrinogen (Fg) with varying Fg concentration ranging from 6, 3 and 1 mg/ml (Sigma Chemical, St. Louis, MO) and cell suspension (2 million cells/ml) or media, were mixed and a 50 μ l aliquot was placed into the center of a 6-well petri-dish. 5 μ l of a 2.5E-3 U/ μ l thrombin solution (Sigma Chemical, St. Louis, MO) was added directly onto the fibrinogen/cell-mixture. Cells used in these studies included DF-1 cells, GFP-DF-1, AS-VEGF:DF-1 and VEGF:DF-1. Polymerization was complete within 15 minutes at 37°C and produced a three-dimensional gel of fibrin entrapping cells or culture media in the center of the dish. As shown in Figure 4 GFP:DF-1 cells are entrapped in a ~~fibrin~~fibrin clot. 3 ml of the culture media supplemented with Polybrene were added to each well after polymerization of fibrin. A 1 ml aliquot was taken out of each well daily for a total of 10 days and replaced with fresh DF-1 culture media. Aliquots were stored at -70°C till assayed by ELISA for p27 and VEGF expression as described earlier. Culturing the cells with varying concentrations of Fg was used to show that virus is released into the culture media and to determine fibrin clot stability over a 10-day culture period.--

Please replace the paragraph beginning at page 26, line 16, with the following rewritten paragraph:

--In one embodiment of the invention, after determining that Matrigel™ successfully entraps cells the viral release of entrapped cells in a Matrigel™ clot can be determined. Experimentally the infectivity of virus carrying gene for GFP entrapped in Matrigel™ was determined. For this determination, 3E5 DF-1 chicken fibroblast cells were plated onto a 6-well plate. Liquid Matrigel™ was mixed with GFP:RCAS viral ~~supernatents~~supernatant at a ratio of 3:2 and 100 µl of Matrigel™/virus ~~supernatents~~supernatant was placed in one well of a 6-well plate. As controls, 100 µl of Matrigel™ was added to wells of a 6-well plate. In addition, there was added 100 µl of Matrigel™/virus ~~supernatents~~supernatant to 0.7 cm² nylon fabric disks and placed nylon containing Matrigel™/virus mixture into wells of a 6-well plate. Nylon fabric with and without addition of 100 µl Matrigel™ served as additional controls in this study. In order to prevent polymerization of Matrigel™ prior to placement into well, Matrigel™ was only handled with ~~pipet~~pipette tips kept on ice. Plates were placed in 37°C incubator and 90% humidity and cells were inspected for green fluorescence daily. After a few days of incubation only DF-1 cells with viral addition showed green fluorescence cells. Hence, virus entrapped in Matrigel™ is still able to infect DF-1 cells.--

Please replace the paragraph beginning at page 30, line 10, with the following rewritten paragraph:

--For histological evaluation of tissue reactions induced in the CAMs, control or test material treated CAMs were fixed *in situ* (10% buffered formalin) at various days post placement of the test sample on the CAMs. The buffered formalin fixed tissue was then processed for paraffin embedding and sectioning. Generally, five µm sections were prepared of the various specimens, mounted on glass slides and stained with hematoxylin and eosin (H&E) for ~~evaluation~~evaluation of histopathology. Histologic evaluation of tissue reactions in the CAMs was done on specimens obtained at 1 day, 4 days and 8 days post placement of the test materials.--

Please replace the paragraph beginning at page 31, line 3, with the following rewritten paragraph:

--It should be noted that histologically the CAM, a transient respiratory organ for the developing chick embryo, consists of a mesodermal stroma lined by an outer ectodermal (air side) and an inner endodermal composed of allantoic epithelium as shown in Figure 8. The ectoderm layer is composed of a microvasculature, which serves primarily for gas exchange, and a chorionic epithelial layer. The mesodermal stroma is composed of a complex vasculature supported by thin collagen fibers and fibroblasts. The ectoderm and endoderm are separated from the mesoderm by basement membranes. The CAM has an approximate thickness of 100 to 200 μm .--

Please replace the paragraph beginning at page 31, line 28, with the following rewritten paragraph:

--In order to evaluate the acute inflammatory response of the *ex ova* CAM, bacterial endotoxins were mixed with India ink, and was placed on top of the chorioallantoic membrane of 7-day-old embryos. The india ink was added to the endotoxin for ease of visualization over the 8 day time course of the study (days 1, 4 and 8 post placement). Gross morphologic evaluation of the CAMs demonstrated that post-placement of 1 to 8 days resulted no detectable gross pathology as shown in Figures 9B and 9C. Figure 9A demonstrates the normal gross morphology of the CAM development of a 12-day-old embryo. Evaluation of H&E stained CAM tissue sections from day 1 post placement of endotoxins/india ink, demonstrated that bacterial endotoxins induced a strong acute inflammatory response, with influx of both plasma proteins (edema) and heterophiles (chick polymorphonuclear leukocytes (PMNs)) into the CAM tissue. Heterophiles are the equivalent to mammalian neutrophils, and are a principal effector cell line of innate host defenses in avian. Generally, the acute inflammation induced by endotoxin/india ink remained localized on or near the surface of the ectoderm as shown in Figure 9F. Hyperplasia of the ectodermal epithelial cells was all seen in the ~~endotoxin/dia~~endotoxin/india ink treated CAMs. A thickening of the CAM occurred at sites of inflammation likely due to tissue edema. The endotoxin/india ink treated CAMs were also evaluated for tissue reactions after 4 days and 8 days post-placement of endotoxin/India ink. By 4 days post-placement of the endotoxin/india ink

histologic evaluation of the CAMs indicated a massive influx of mononuclear leukocytes (monocytes and lymphocyte) into the ectoderm layer of the CAM as shown in Figure 9G. After 8 days post-placement of the endotoxin/India ink, the inflammation displayed significant resolution, as shown in Figure 9H. By day 8 post-placement (PP), the inflammation appeared to be resolving on the surface of the CAM. The Histology of normal CAM is provided for comparison as shown in Figure 9E.--

Please replace the paragraph beginning at page 40, line 31, with the following rewritten paragraph:

--Analysis of the CAM tissue homogenates demonstrated that CAM tissue from the mVEGF:DF-1 treated CAMs had significant mVEGF content (137.6 ± 3.67 pgs of mVEGF per mg total protein), that none of the CAM tissue from media or control DF-1 cells (DF-1, mVEGF antisense:DF-1 or GFP:DF-1 Cells) had detectable mVEGF in the tissue homogenates. It should be noted that the ELISA detected only mouse mVEGF and not chicken mVEGF. Thus, these experimental studies are evaluating only the murine VEGF gene product expressed in DF-1 cells by gene transfer and show that the mouse VEGF gene that was inside the genetically engineered cells used for this ~~experimenta~~experimental gene therapy to induce new blood vessel was expressed in vivo in the chicken CAM, and that its expression was only seen when there was new blood vessel formation.--

Please replace the paragraph beginning at page 41, line 10, with the following rewritten paragraph:

--One embodiment of the present ~~invention~~invention includes the use of fibrin for the delivery of genes in the *Ex Ova* model. After experimentally showing that fibrin clots are not only able to entrap cells but also to release the virus, as discussed above, the effect of neovascularization of VEGF:DF-1 cells entrapped in a fibrin clot in the *ex ova* chick model was determined. For this experimental study, VEGF:DF-1 cells or control cells (DF-1, GFP:DF-1 and AS-VEGF:DF-1) (2 million cells/ml) were mixed with

equal amounts of a physiological Fibrinogen (Fg) solution (3 mg/ml) and a 50 μ l aliquot was placed onto a nylon. After 5 μ l of a $2.5E-3$ U/ μ l thrombin solution (Sigma Chemical, St. Louis, MO) was added directly onto the Fg/cell-nylon or Fg/media-nylon mixture, the various nylon-disks were placed in a tissue culture incubator for about 15 minutes at 37°C for polymerization of the fibrin to occur. Disks were lifted out of the petri-dish, and care was taken to make sure most of the fibrin clot entrapping the cells or media was still attached to the nylon. The fibrin clot was then placed with the fibrin site down on top of the CAM of an 8-day old chicken embryo. Thus, it was experimentally shown how to make an ATS which includes a matrix material and includes genetically engineered cell(s). Also shown is how the ATS can be used in ~~conjunction~~conjunction with a biomaterial support for such things a gene therapy, for example, for the induction of new blood vessels in vivo in the ex ova CAM model system.--

Please replace the paragraph beginning at page 43, line 1, with the following rewritten paragraph:

--One embodiment ~~for~~of the present invention includes preparation sensor-Cell-Placement on CAM. Experimentally, after sterilizing the sensors by, for example, overnight UV exposure, sensors were dip-coated in egg-white (EW) to enhance cell attachment. The sensors were placed into a 60x15 mm tissue culture treated petri-dish and after the EW was dried out, a fibrin clot containing either media or the cell suspensions (mVEGF:DF-1 or GFP:DF-1) was formed on top of the sensor loop. The formation of fibrin clots served as a matrix material to keep the cells localized around the sensor. Briefly, equal amounts of human fibrinogen (6 mg/ml; Sigma Chemical, St. Louis, MO) and cell suspension (2 million cells/ml) or media were mixed and a 50 μ l aliquot was placed onto the sensor loop. 5 μ l of a $2.5E-3$ U/ μ l thrombin solution (Sigma Chemical, St. Louis, MO) was added directly onto the fibrinogen/cell or fibrinogen/media mixture. Polymerization was complete within 15 minutes at 37°C and produced a three-dimensional gel of fibrin entrapping cells and sensor or culture media and sensor. Sensors were lifted out of the petri-dish, and care was taken to make sure most of the

fibrin clot entrapping the cells was still attached to the sensor. The sensor was then placed on top of the CAM of an 8-day old chicken embryo.--

Please replace the paragraph beginning at page 43, line 17, with the following new paragraph:

--Sensor performance *in vivo* was conducted 6 to 10 days post-placement of the sensor using the three-electrode system described above. The petri-dish containing the developing chicken embryo was placed into a sand box, which was kept at 38°C and a potential of 700 mV was applied to the working electrode. After stabilization of the background current, 200 µl of a 50 mM acetaminophen solution (in PBS) was injected i.v. and the sensitivity to acetaminophen was determined and recorded onto a chart recorder (Bioanalytical Systems (BAS), West Lafayette, IN). The sensor current was monitored for approximately 20 minutes before another i.v. injection of acetaminophen was performed. Sensor response to acetaminophen was calculated as nano Amperes (nA) of the initial current increase. Thus, a method was experimentally shown for evaluating the function of a chemical sensor, for example an acetaminophen ~~sensore~~sensor and for evaluation an ATS-sensor combination, wherein the ATS contains genetically engineered cell(s) and a matrix material such as fibrin.--

Please replace the paragraph beginning at page 43, line 30, with the following rewritten paragraph:

--Impact of mVEGF Gene Transfer on Loop-Type Sensor Function *in Vivo* was then demonstrated experimentally. The focus of this experimental study was to: 1) demonstrate the *in vivo* function of the acetaminophen sensors, and 2) to determine the impact of mVEGF induced increase of vessel density surrounding the sensor on sensor function *in vivo*. In general, sensors were incorporated after only a few days post-placement and only sensors completely incorporated were finally utilized to compare responses between control sensors and angiogenesis induced around sensor. After 6-days to 10-days post-placement, sensors were tested using the 3 electrode system

described in the methods section above. Sensors implanted on CAMs with buffer or GFP:DF-1 cells displayed no induced neovascularization around the sensor as shown in Figure 19. In addition, minimal sensor responses to i.v. acetaminophen injection were determined as shown in Figure 20 wherein the results were media: 133.33 ± 27.64 nA (n=6); GFP:DF-1: 187.50 ± 55.43 nA (n=6). In addition, it was observed that the sensors implanted with mVEGF:DF-1 cells displayed massive neovascularization as shown in Figure 19B. Also observed was a massive sensor response to i.v. injected acetaminophen as shown in Figure 20 wherein the mVEGF:DF-1 results were 1387.50 ± 276.42 nA (n=6)). Statistical analysis indicated that there was no statistical difference in sensor response between media treated sensors and sensors treated with GFP:DF-1 cells. However, when the responses of media treated sensors or GFP:DF-1 treated sensors were compared to mVEGF:DF-1 treated sensors there was major statistical significance ($p < 0.001$). As experimentally has been shown, the VEGF-GE-Cell-ATS dramatically enhanced the in vivo function of the Chemical sensor in vivo, with the dramatic increase in sensor function being associated with the massive ~~neovascularization~~neovascularization that occurred only at the site of the VEGF-GE-Cell-ATS-sensor implantation. Thus, ATS with genetic engineered cell(s) and matrix material such as Fibrin enhance both neovascularization and sensor function at sites of ATS-sensor implantation.--

Please replace the paragraph beginning at page 61, line 17, with the following rewritten paragraph:

--One embodiment of the present invention includes using VEGF:DF-1 to induce neovascularization in immunodeficient mice (nude/nude). Experimentally In order to determine if chicken VEGF:DF-1 cells entrapped in ATS system (Matrigel™) are capable of inducing neovascularization in ~~mice~~mice, VEGF:DF-1 cells were suspended in Matrigel™ and injected s.q. in the back of the mice. Mice were sacrificed 8 days later, and the injected tissue was removed, fixed and processed for H&E staining. Mice injected with DF-1/antisense VEGF(AS-VEGF:DF-1) cells (controls), displayed no neovascularization around the Matrigel™ as shown in Figure 41B. In contrast, mice

injected with VEGF:DF-1 cells displayed a robust neovascularization around the Matrigel™ as shown in Figure 41A), and in many cases the neovascularization penetrated the interior of the Matrigel™ as shown in Figure 41C, note that the arrows point to microvessels and indicate a colony of VEGF:DF-1 cells. It was also noted that tissue inflammation was minimal around the implanted ATS, and when present it did occur at the margins of the ATS, it did not penetrate into the ATS. This ability of the ATS system to 1) cause little tissue inflammation when implanted *in vivo* and 2) to block access of the inflammatory cells (i.e. inflammatory cells of innate immunity such as PMNs and macrophages) to the engineered cells in the ATS, resulted in protection of the cells within the ATS and enhancements of cell viability and function *in vivo*. Finally, it was observed that when the genetically engineered VEGF-DF-1 cells were injected into the skin of the mice without Matrigel™ the cells failed to induce any neovascularization. This clearly indicates the critical role of the Matrigel™ basement membrane in both promoting and protecting cells implanted at tissue sites, and underscores the importance of the matrix in both protecting the sensor as well as promoting cell survival and function at the site of ATS-sensor implantation. This experimental example shows that an ATS system composed of genetically engineered cells (chicken cells which overproduce the angiogenic factor VEGF) into an immunodeficient mouse (nude/nude) in a Matrigel™; this ATS system induces neovascularization into the ATS system only when the cells engineered to overproduce VEGF are in the ATS but not when control (VEGF anti-sense) engineered cells are in the ATS. Additionally, this experimental example demonstrates that the ATS induced minimal tissue inflammation when implanted and that basement membrane matrix (Matrigel™) appears to protect the engineered cells from destruction by the tissue inflammation, because the cells within the Matrigel™ were viable. Finally, this experimental example shows that Matrigel™ ATS is required to promote efficient neovascularization; e. g. cells injected into the skin of the mice without matrigel did not induce neovascularization, thus indicating the important role of the matrix material, for example basement membranes, in the ATS in cell viability *in vivo*.--

Please replace the paragraph beginning at page 66, line 25, with the following rewritten paragraph:

--For example, as shown in Figure 42, a prophetic experiment includes an adenovector based gene transfer in cells that contain little or no CAR can be achieved by first transferring the CAR gene into the CAR deficient cell, demonstrating CAR expression, and finally transferring the gene of choice into the CAR transfected cell. To achieve this can first culture NIH 3T3 mouse fibroblasts at a concentration of 3E5 cells. Supercoiled plasmid containing mouse CAR or human_CAR genes is then added to the cells in DMEM containing lipofectamine and incubated at room temperature. Next, the cells-plasmid-lipofectamine culture is transferred to 37°C and 5% CO₂ for 5 hours. After the 5 hr time frame, 2 ml of 10% FBS is added to the culture and it is incubated at 37°C and 5% CO₂. CAR expression in the cells is then determined by immunocytochemistry and western blot technology using an antibody that is specific for mouseCAR (mCAR). The resulting mCAR positive cells can be tested for gene transfer using adenoviral vectors containing green fluorescence protein gene (GFP), human VEGF genes or mouse VEGF genes by incubation of the CAR positive 3T3 fibroblast with these viral vectors individually. The resulting transfected cells can be tested for successful gene transfer by 1) evaluating the GFP transfected cells for green fluorescence appearance under direct microscopic evaluation; 2) evaluating expression of mouse VEGF expression by immunoassays (ELISA and Western blot) using an antibody specific for mouse VEGF; 3) evaluating expression of human VEGF expression by immunoassays (ELISA and Western blot) using an antibody specific for human VEGF. The resulting mouse VEGF or human VEGF 3T3 fibroblast can then be added to matrigel and inject s.q. in the skin of mice to determine there ability to induce new blood vessel formation using standard histological evaluation of the tissue obtained from the site of implantation. The 3T3 cells that successfully induce new blood vessel formation in the mouse skin can then be used in the ATS with the implantable glucose sensor to determine the ability of these genetically engineered cells to enhance glucose sensor function *in vivo*.--